ond, we cannot say unequivocally that the somatic cell nucleus is reprogrammed as it coexists with the original chromosomes from the ESC in the hybrid cell. To be sure, the ESC chromosomes need to be eliminated from the hybrid cell to see if the somatic cell nucleus continues to display its new identity. Of course, being able to remove the ESC chromosomes while retaining the reprogrammed somatic nucleus would make this procedure extremely valuable even if we do not understand the mechanism. However, this is not possible at present. Nevertheless, the likelihood that hESCs have the potential to reprogram somatic cell nuclei is a big step forward because it means that there is a large volume of biological material available for biochemical characterization of the reprogramming factors present in these cells. There is also the potential to develop cell-based assays to hunt for these key reprogramming factors.

But we also need to know the nature of the reprogramming factors themselves. A key requirement during reprogramming is the efficient erasure of existing epigenetic modifications associated with DNA and histones. Are oocytes and ESCs equivalent in this respect? Some studies suggest that certain forms of histone modifications may be erased more efficiently in the pluripotent epiblast compared to oocytes (Bao et al., 2005; Santos et al., 2003). If ESCs inherit this property from epiblast cells from which they are derived, then this could make ESCs in some respects more efficient at reprogramming somatic cells. The recent identification of a histone demethylase called LSD1 (Lee et al., 2005; Metzger et al., 2005) could be an important step forward if this or similar molecules have a role in nuclear reprogramming. Indeed, we need to understand the mechanism of nuclear reprogramming in much greater detail, and the Cowan et al. study is certainly a step in this direction.

M. Azim Surani
Wellcome Trust Cancer Research UK Gurdon Institute
University of Cambridge
Tennis Court Road
Cambridge CB2 1QN
United Kingdom

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Taking LSD1 to a New High

Histone modifications mediate changes in gene expression by altering the underlying chromatin structure or by serving as a binding platform to recruit other proteins. One such modification, histone methylation, was thought to be irreversible until last year when Shi and coworkers broke new ground with their discovery of a lysine-specific histone demethylase (LSD1). They showed that LSD1, a nuclear amine oxidase homolog, is a bona fide histone H3 lysine 4 demethylase (Shi et al., 2004). Now, a new study from Shi et al. (2005) published in a recent issue of *Molecular Cell*, together with two studies recently published by Metzger et al. (2005) and Lee et al. (2005) in *Nature*, reveal that LSD1's specificity and activity is in fact regulated by associated protein cofactors.

Development, cell proliferation, and cellular responses to environmental signals are all orchestrated by coordinated patterns of gene expression. DNA-encoded genetic information is packaged into a chromatin polymer, which must be opened to allow increased accessibility for gene regulatory factors, or compacted to restrict access of the transcriptional machinery to target genes. In general, these "opened" and "closed" chromatin states correspond to gene activation versus gene repression, respectively.

What are the molecular switches that govern these opposing states as cells attempt to activate some genes while repressing others? A rich history of chromatin research has provided longstanding clues as to what form these switches might take, and the last decade has transformed those early clues into hard evidence. Histone modifications, such as acetylation, phosphorylation, and methylation, are the switches that alter chromatin structure or form a binding platform for downstream "effector" proteins to allow transcriptional activation or repression. Histone acetylation is the best characterized histone modification. A steady-state balance between histone acetylation and deacetylation and hence the transcriptional competency of target genes depends on the interplay of opposing enzymatic activities: forward reactions catalyzed by histone acetyltransferases and reverse reactions catalyzed by histone deacetylases. Exciting recent findings are rapidly extending these lessons to another form of chromatin modification, histone methylation.

About five years ago, the first methyl lysine-adding enzymatic activity was identified using histone H3 as a substrate (Rea et al., 2000). However, methylation of histones was thought to be different from acetylation or phosphorylation in that it was not reversible due to the high thermodynamic stability of the N-CH_3 bond.

Once placed, the methyl mark was thought to be static, whether promoting activation of gene expression, for example, through methylation of lysine 4 (K4) on histone H3 (see Figure 1A), or repressing gene expression through methylation of lysine 9 (K9) on histone H3 (Figure 1B). Not only does the particular "site" of methylation matter, but also the methylation "state" because methylated lysine can come in multiple flavors (mono-, di-, and trimethyl). Thus, as is often the case in biology, the devil is in the details, and with histone methylation, every detail is likely to be critical for the biological outcome.

Although most research has focused on the role of histone modifications, such as acetylation and methylation in gene regulation and other epigenetic phenomena, there is increasing recognition that histone modifications are an important component of human disease, notably cancer. A recent study reported the potential application of global changes in specific histone acetylation and methylation marks as predictors of clinical outcome for certain low-grade prostate cancers (Seligson et al., 2005). The importance of lysine methylation in human disease is also underscored by the wide range of different methyltransferases that correlate with carcinogenesis when mutated (Schneider et al., 2002).

Last year, Shi and coworkers turned the world of histone methylation upside down. They presented evidence that LSD1, a nuclear amine oxidase homolog, is a bona fide histone lysine demethylase (Shi et al., 2004). LSD1 is highly conserved between organisms ranging from the fission yeast *Saccharomyces pombe* to humans. It contains a carboxyl-terminal amine oxidase domain as well as a centrally located SWIRM domain, a protein-protein interaction motif found in multiple chromatin-associated proteins. The oxidation reaction catalyzed by LSD1 is dependent on the cofactor flavin adenine dinucleotide (FAD) and generates an unmodified lysine and a formaldehyde byproduct at the end of its catalytic cycle (Shi et al., 2004). Remarkably, recombinant LSD1 is highly specific for mono- and dimethylated H3 K4 (see Figure 2, upper panel), whereas other known major methylation sites in the tails of H3 and H4 histones are not its substrates. Demethylation of trimethylated lysine, however, is prevented by the absence of a protonated nitrogen required for oxidation. Consistent with its role in removal of an "active" methylation mark thereby promoting repression of gene expression, LSD1 is found in corepressor complexes (Shi et al., 2004). As a component of these complexes, LSD1 associates with other histone-modifying enzymes such as histone deacetylases and methyltransferases. In concert with transcriptional repression, loss of LSD1 function leads to derepression of genes regulated by CoREST, a protein involved in gene silencing and a member of the LSD1 complex (Shi et al., 2004).

Although the discovery of the first lysine demethylase represents a major breakthrough in chromatin biology, it also raises many questions. For example, how can the regulatory complexity of histone demethylation be accomplished with only ten LSD1-related proteins (Kubicek and Jenuwein, 2004)? For comparison, the corresponding regulation of histone methylation requires over 70 proteins that contain the specific motif (SET domain) responsible for methylation. One possibility is that the specificity and activity of LSD1 can be modulated by association with specific cofactors. Three recent papers demonstrate that this scenario is indeed likely (Lee et al., 2005; Metzger et al., 2005; Shi et al., 2005) and also reinforce the general view that histone methylation is a reversible modification with far-reaching implications for human disease.

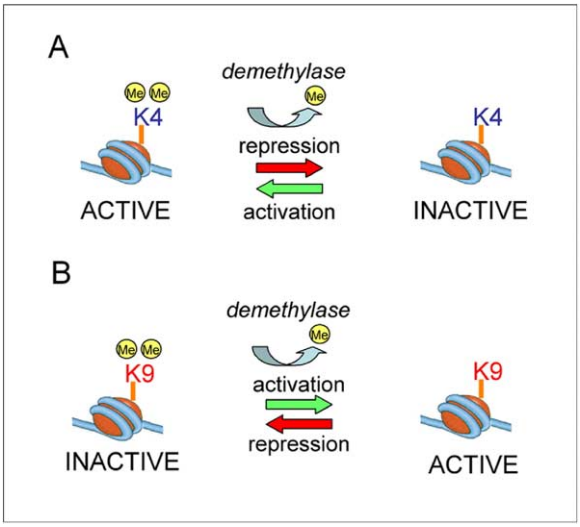

Figure 1. The Methylation of Histone Lysines Is Reversible and Regulates Gene Expression

(A) Methylation of H3 K4 is often associated with active genes, and conversely, its demethylation accompanies gene repression.
(B) In contrast, methylation of H3 K9 is often associated with silenced genes, hence removal of H3 K9 methyl marks coincides with gene activation.

Metzger et al. (2005) now report in *Nature* that they have isolated LSD1 in a search for new proteins associated with the androgen receptor. Androgen receptor belongs to a nuclear-receptor family of proteins that, upon binding of ligand, regulates expression of genes containing androgen-response DNA elements. Increased expression of androgen receptor and activation of its target genes appears to be an important mechanism for the conversion of metastatic prostate cancer into a treatment-resistant form (Chen et al., 2004). Metzger et al. (2005) provide evidence that demethylation of lysine residues could also have important implications for this process. LSD1 directly associates with the androgen receptor and, surprisingly, acts as a coactivator for transcriptional activation by the androgen receptor. Binding of ligand to the androgen receptor coincides with the loss of the repressive H3 K9 methylation from the androgen-response DNA element but, interestingly, has no effect on H3 K4 methylation (see Figure 2, bottom panel). Using pargyline as an inhibitor of the amine oxidase activity of LSD1 and gene knockdown through RNA interference, the authors demonstrate that LSD1 is responsible for demethylation of the mono- and dimethyl forms of H3 K9. Furthermore, LSD1 is required for transcriptional activation mediated by the androgen receptor, but not that mediated by the estrogen receptor or retinoic acid receptor. LSD1 affinity-purified in the presence, but not in the

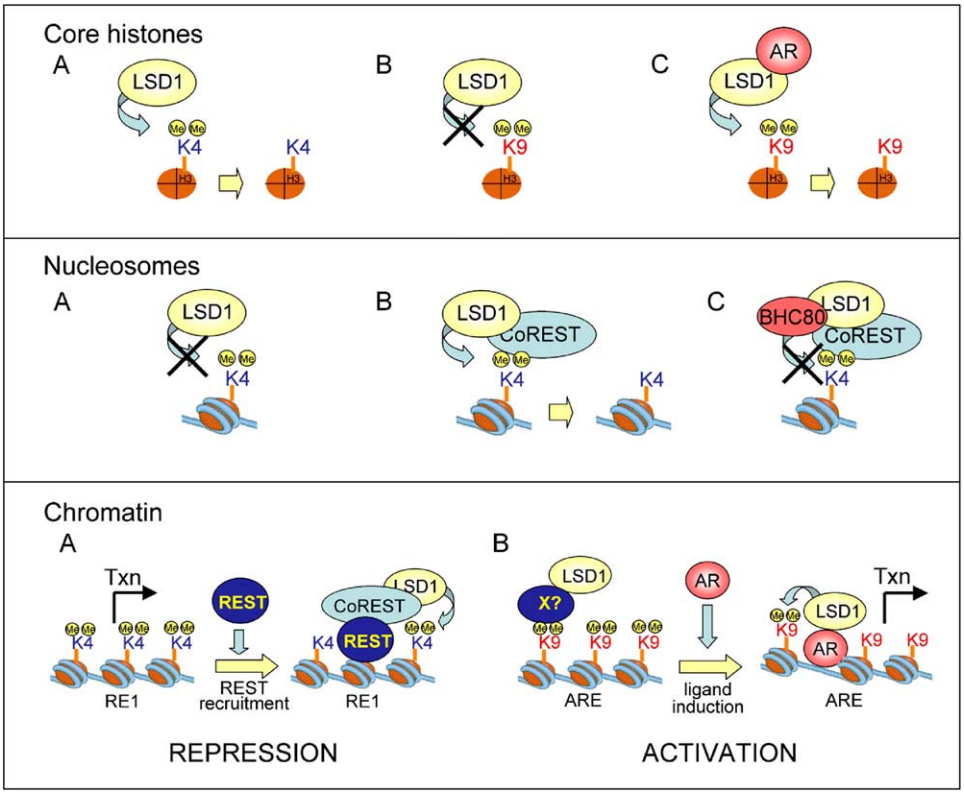

Figure 2. Regulation of LSD1 Specificity by Association with Protein Cofactors

(Upper panel) Recombinant LSD1 specifically demethylates H3 K4 of core histones (A), but not H3 K9 (B) (Shi et al., 2004). (C) LSD1 demethylates H3 K9 when affinity purified in the presence of the androgen receptor (AR; Metzger et al., 2005).

(Middle panel) (A) Recombinant LSD1 alone does not bind to or act upon nucleosomal substrates. (B) Association of LSD1 with CoREST endows LSD1 with the ability to demethylate H3 K4 on nucleosomal substrates (Lee et al., 2005; Shi et al., 2005). (C) BHC80 inhibits the demethylation of nucleosomes mediated by the LSD1-CoREST complex (Shi et al., 2005).

(Lower panel) (A) LSD1, as part of the CoREST corepressor complex, is recruited to genes containing the REST-responsive repressor element 1 (RE1) and participates in gene silencing through demethylation of H3 K4 (Shi et al., 2004). (B) Ligand-induced recruitment of androgen receptor (AR) to the androgen receptor element (ARE) is accompanied by demethylation of H3 K9 by LSD1, leading to transcriptional activation (Metzger et al., 2005). However, LSD1 is already tethered to the ARE through an unknown mechanism (indicated by factor X) prior to the induction of gene expression by the androgen ligand.

absence, of androgen receptor can demethylate H3 K9. This suggests that the androgen receptor itself or alternatively one of its associated proteins is able to modulate the specificity of LSD1 (Figure 2, top panel). Thus, this study shows a new, cofactor-regulated specificity for histone demethylation and demonstrates that LSD1 can act as a transcriptional coactivator. It also shows that monoamine oxidase inhibitors, such as pargyline, can inhibit LSD1, which may prove useful in the studies of histone demethylases and have important therapeutic implications for prostate cancer.

Several important questions remain to be addressed. For example, it remains unclear whether the purified LSD-androgen receptor complex fails to use methylated H3 K4 as a substrate in vitro. Also, association of LSD1 with androgen-response DNA elements is independent of the presence of androgen receptor at the promoter. How then is LSD1 tethered to androgen-response DNA elements if it is not directly recruited by the androgen receptor? In one possible scenario, LSD1 may be present at the promoter in a repressive complex prior to induction of gene expression by a ligand (Figure 2, bottom panel). Once the androgen receptor interacts with its ligand, this complex associates with LSD1, which may alter its specificity, thereby converting it from a repressor of gene expression into an activator. However, reduced lysine 4 methylation is not observed when LSD1 is bound in the absence of androgen receptor, indicating that bound LSD1 may be present in an enzymatically inactive conformation. Further studies to investigate the presence or absence of LSD1-associated repressor complexes at androgen receptor targets may shed light on these issues. Another interesting and unexplained observation is that H3 K9 trimethylation appears to be altered upon activation of the androgen receptor by its ligand. This effect is independent of LSD1, consistent with the inability of FAD-dependent amine oxidases to use trimethylated lysines as substrates. What enzymatic activities are responsible for loss of H3 K9 trimethylation upon activation of the androgen receptor? Finally, it will be essential to determine the structural basis of the substrate recognition

by LSD1 in the absence and presence of androgen receptor.

The studies by Lee et al. (2005) and Shi et al. (2005) address the role of cofactor association in the regulation of LSD1 activity in transcriptional repression through demethylation of H3 at the K4 position. Although insights into this regulation are certainly of great interest, such regulatory mechanisms are not unprecedented in chromatin biology. In the early days of acetylation, recombinant catalytic enzymes were found to behave quite differently from the multisubunit complexes that contained them (Grant and Berger, 1999). Moreover, the form of the substrate was critical, as core histone substrates were affected differently from nucleosomal substrates in a manner often dependent on association with other proteins. Now, work by two groups demonstrates that this mode of regulation can be extended to histone demethylases, as activity of LSD1 toward nucleosomes is regulated by a cofactor (Lee et al., 2005, Shi et al., 2005). Both groups focused their attention on CoREST, a member of an LSD1-containing complex, whose components are well defined (Hakimi et al., 2002). CoREST is recruited by the silencer REST to repress transcription of neuronal genes (Hakimi et al., 2002; see Figure 2, bottom panel). Through in vitro reconstitution of the complex, Lee et al. (2005) functionally dissect the role of the polypeptides present in the CoREST complex. In a complementary study, Shi et al. (2005) show that two members of the LSD1 complex, CoREST and BHC80, have opposing effects on LSD1 activity. Both groups demonstrate that CoREST endows LSD1 with the ability to associate with and demethylate nucleosomal substrates (see Figure 2, middle panel). Lee et al. (2005) conclude that the presence of either one of the two SANT domains of CoREST, a protein motif present in many chromatin-associated proteins, is necessary for its association with LSD1 and for demethylation of nucleosomes. In contrast, Shi et al. (2005) demonstrate that a region situated toward the amino terminal of the second SANT domain of CoREST binds to LSD1 and that, in conjunction with this binding domain, the second SANT domain alone is sufficient for the stimulation of LSD1 enzymatic activity. Whatever the case, these results suggest a mechanism whereby CoREST binds to LSD1 and tethers it to the nucleosome, bringing the amine oxidase domain close to the H3 tail. Both groups show that CoREST knockdown through small interfering RNAs results in the increase of H3 K4 dimethylation at the REST-dependent promoter, underscoring the importance of CoREST as a cofactor for the histone demethylase. Shi et al. (2005), however, delve deeper into a possible regulatory mechanism by making the interesting observation that CoREST is required not only for the activity, but also for the stability of LSD1, in a proteasome-pathway-dependent manner. This group also showed that another component of the CoREST complex, BHC80, inhibits demethylation of nucleosomes mediated by the LSD1-CoREST complex. This adds to the potential complexity of the regulation of LSD1 activity within the CoREST complex (Shi et al., 2005; see Figure 2, middle panel).

Perhaps the most important discrepancy between the results described by Lee et al. (2005) compared with those described by Shi et al. (2005) is related to the involvement of histone deacetylation in the activity of LSD1. Lee et al. (2005) argue that the demethylase activity of the CoREST complex is unaffected by inhibition of the histone deacetylases present in the complex, suggesting that the demethylation reaction does not require prior deacetylation and that LSD1 can act upon acetylated substrates. In contrast, Shi et al. (2005) show that hypoacetylated nucleosomes are more susceptible substrates for demethylation than their hyperacteylated counterparts. The authors also note that the SANT domain of another corepressor, SMRT, binds preferentially to hypoacetylated histone tails (Yu et al., 2003). Thus, the results described by Shi et al. (2005) suggest a model whereby deacetylation of nucleosomes by histone deacetylases sets the stage for demethylation, possibly by allowing for tighter binding of the CoREST SANT domain to the nucleosomes, which in turn results in increased recruitment of LSD1 to nucleosomal substrates. Although discrepancies between the two papers need to be resolved, given the fact that LSD1 is accompanied by histone deacetylases in various protein complexes, and mindful of lessons learned from other histone modifications, we think that crosstalk between the deacetylation and demethylation pathways is very plausible.

Together, these three studies add to the continually evolving world of histone modifications in general and lysine demethylation in particular. Clearly, there is much gold left to be mined from the study of histone methylation and the enzyme systems responsible for the balance between methylation and demethylation. Although new evidence suggests that histone methylation marks may not be as stable as was first thought, we conclude that histone demethylation is here to stay.

Joanna Wysocka, Thomas A. Milne, and C. David Allis
Laboratory of Chromatin Biology
The Rockefeller University
New York, New York 10021

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